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THE EFFECTS OF ASCORBIC AND GIBBERELLIC ACID ON METABOLISM OF WHEAT (*TRITICUM AESTIVUM L.*) AT SEEDLING STAGE UNDER SALINE CONDITIONS

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ABSTRACT

The effects of hormonal priming with ascorbic acid and gibberellic acid on wheat (Triticum aestivum L.) metabolism during germination phase, under saline conditions were studied to determine their effectiveness in increasing relative salt-tolerance. Four replications of seeds of wheat var. GW- 496 were pre-soaked in three levels each of ascorbic acid (AsA) viz., (50, 100 and 150 mg L^{1}) and gibberellic acid (GA_3) viz., (150, 200 and 250 mg L^{-1}) for 2 hrs under salinity stress given by treating with NaCl @ 50, 75 and 100 mm. Seeds primed with 100 mg L^{-1} AsA and 250 mg L^{-1} GA_3 effectively enhances seed moisture content, root length, shoot length, root shoot length ratio and vigour index. Among the biochemical parameters proline content was enhanced by the application of 100 mg L^{-1} AsA and 150 mg L^{-1} GA_3. Seeds primed with 100 and 150 mg L^{-1} AsA and 250 mg L^{-1} GA_3 mostly enhanced the activity of polyphenol oxidase, peroxidase and catalase. It could be concluded that, pretreatment of wheat cultivar with AsA and GA_3 could partially alleviate the harmful effect of salinity by increasing water and nutrient use efficiency, antioxidant activity and accumulation of osmolytes.

***KEYWORDS:** Ascorbic Acid, Gibberellic Acid, Physiological Parameters, Biochemical Parameters, Proline, Free Amino Acid, Total Soluble Sugar, Polyphenol Oxidase, Peroxidase and Catalase*

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Original Article

INTRODUCTION

Wheat is the second most important staple food crop next to rice, consumed by nearly 35% of the world population and providing 20% of the total food calories. The total cultivated area of wheat in the world is around 221.12 million hectares with a production of 697.8 million tonnes and productivity of 3160 kg/ha MOA. (2013). It occupies about 32% of the total acreage under cereals in the world. Approximately 7% of worlds land area, 20% of the worlds cultivated land and nearly 50 % of the irrigated land is affected by salt stress as reported by Abdelfattah *et al* (2009). In India, total salt affected area is 12 million ha Abdul *et al* (1973). The effect of salinity on plant may cause disturbance in plant metabolism as reported by El-Tayeb *et al* (2005). It was also reported that seed germination, one of the most critical phases in plant life, is greatly affected by salinity Abo-Kassem (2007), which either induces a state of dormancy at low levels or completely inhibits germination at higher levels Iqbal *et al* (2006). Presoaking of seed with optimal concentration of phytohormones and antioxidant has been shown to be beneficial for growth and yield improvement of wheat crop under saline conditions as reported by Mohsen *et al* (2014). Ascorbic acid (AsA) is regarded as one of the most effective growth regulators against abiotic stresses Batool *el al.,* (2012). Azooz *et al* (2013) showed that application of ascorbic acid through seed soaking enhanced

plants growth by increased germination percentage, root and shoot fresh and dry weights, chlorophyll content and higher accumulation osmolytes. Experimental studies on different plants have shown that pretreatment with AsA reduced salt induced adverse effects and resulted in a significant increment of growth and yield Batool *el al.,* (2012). Gibberellic Acid (GA_3) is the most important growth hormone which increases cell growth and elongation, cell division in cambial zone, breaks seed dormancy, promotes seed germination, intermodal length, hypocotyls growth, increases the size of leaves, enable greater photosynthesis and plant metabolism and ultimately increases plant or crop yield under normal as well stress condition. GA_3 is used to revive the plants suffering from salt stress by overcoming the adverse effects of the salt stress on germination Amal and Heba (2014). Hence, the present investigation was carried out in order to investigate the extent of effectiveness of these two growth stimulators in ameliorating the adverse effect of salinity stress.

MATERIALS AND METHODS

The experiment was carried out with four replications, 100 (25 seeds in each replica) seeds of wheat cultivar GW-496 were soaked in distilled water (control), three concentrations of ascorbic acid (AsA) viz., (50, 100 and 150 mg L^{-1}) and three concentrations of gibberellic acid (GA_3) viz., (150, 200 and 250 mg L^{-1}) solutions for 2 hrs and then the same were treated with 2.5 g/l thiram for about 2 minutes. For germination, 25 seeds from each sample were spread in Petri Dishes over Whatman No.1 filter paper. The sufficient volume (10 ml from 1st to 5th day and 20 ml from 5th to 11^{th}) of NaCl concentrations (50 mM, 75 mM and 100 mM) were added to induce salinity stress, whereas distilled water was provided as control. Data analysis was performed using the software "DSAASTAT" statistical software (Version 1.101). Mean separations were performed by Duncan's Multiple Range Test (DMRT) at 5% level.

The Physiological Parameters Recorded were

Seed moisture (%) was recorded at 24 h, Root length (cm), Shoot length (cm), Seedling length (cm) and Root / shoot length ratio were recorded at 11^{th} day after germination. Vigour index- I was also recorded at 11^{th} day after germination as per procedure prescribed by Abdul-Baki and Anderson (1973).

Biochemical Parameters Recorded at 11^{th} Day after Germination were

Proline content was analyzed by the method suggested by Bates *et al* (1973), Polyphenol oxidase and catalase activities were determined by the methods suggested by Malik and Singh (1980). Peroxidase activity was determined by the methods given by Gulibault (1976).

RESULT AND DISCUSSIONS

Seed Moisture Percentage

It sharply decreased with increasing salinity levels. The higher concentration showed more reduction (24%) as compared to mild and moderate concentrations with (7%) and (13%) reduction respectively, as compared to control. The result is in agreement with *El Goumi et al (2011)* and *Shamsi and Kobraee (2013).* Further increase in seed moisture percentage was found after pre-treatment with ascorbic acid and gibberellic acid under saline condition. The highest seed moisture content of AsA treated seeds were found with its moderate concentration. Similar result was reported by *Azooz et al (2013)*. With respect to the treatment of wheat seeds with GA_3, its highest concentration showed greatest seed moisture content. *Ozhan and Hajibabaei (2013)* also showed that interstitial water content of wheat seeds significantly increased when treated with different levels of GA_3 (150, 200 and 250 mg L^{-1}). The result may be due to the fact that hormones generally decrease the viscosity of cytoplasm and increase diffusion of water into the cell.

Root Length

NaCl, at all the concentrations *viz.,* mild, moderate and severe drastically reduced root length at the tune of (37%), (56%) and (72%) respectively. The finding was supported by *Bahrani and Pourreza (2012)*. Further, seeds treated with AsA and GA_3 remarkably increased the root length under saline condition. Seeds treated with highest concentration of AsA were found best under all salinity levels. This result is also supported by *Mohammed (2007)* in bean and *Jamil and Rha (2007)* in sugar beet. The highest concentration of GA_3 here found best for increasing root length under all salinity levels. The result was supported by *Bahrani and Pourreza (2012)* and *Nasri et al (2012)*. The production of high root length in plant raised from seeds treated with these growth promoting substances suggested that the rate of absorption of available nutrients might have significantly been enhanced by the pretreatment with GA_3 and AsA.

Shoot Length

Treatment of NaCl, at the moderate and severe stress condition drastically reduced shoot length with a percentage reduction of (43%) and (62%) respectively, the reduction was a little less (30%) with the mild stress condition. Shoot length decreased progressively with increase in salinity *(Bahrani and Pourreza, 2012)*. AsA and GA_3 application helped the salinity-challenged plants to a different degree in the reversal of altered growth and drastically increased the seedling shoot length. It was observed that under all three stress conditions seeds treated with AsA @ 100 mg L^{-1} were most favorable. Similar observation was reported by *Behairy et al (2012)*. This result may be due to increased auxin concentration in response to AsA application, with the resultant stimulation in cell division and cell enlargement under saline condition *(Khan et al 2011*). Taking in view the seeds treated with GA_3 under mild and severe stress conditions the maximum shoot length was exhibited at concentration of 250 mg L^{-1}. Such trend of increased shoot length by the application of GA_3 under salinity stress was reported by *Ghobadi et al (2012) and Jasmine and Merina (2012)*.

Root to Shoot Length Ratio

A progressive increase in root to shoot length ratio was found with increase in salinity levels. Severe stress forced more increase of root/shoot length ratio with the tune of (10%), as compared to moderate (22%) and mild stress (25%) conditions. The result is in accordance with *Hameed et al (2008).* Application of AsA and GA_3 were found to increase the root to shoot length ratio under salinity stress levels. For the seeds pretreated with AsA under all stress levels, the maximum increment was found with the application of highest level of AsA. *Rafique et al (2011)* also reported that application of 15 mg L^{-1} AsA showed enhanced root/shoot length ratio under saline condition. Under mild and moderate stress condition, GA_3 @ 150 and 250 mg L^{-1} were effective in nullifying the adverse effect of salt stress on root to shoot length ratio. However, under severe stress GA_3 @ 200 mg L^{-1} showed best result. This result was justified by the fact that both AsA and GA_3 regulates physiological and biochemical activities in plants and can be used as a potential growth promoter to improve root growth more than the shoot growth under saline conditions, thereby increasing root to shoot length ratio.

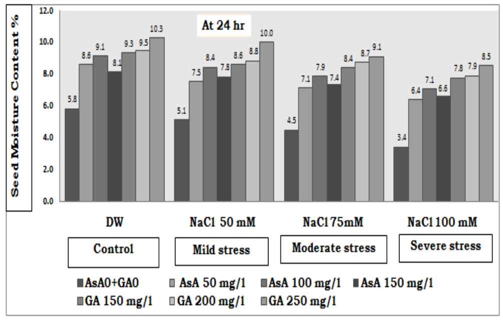

Figure 1: Effect of Nacl Induced Salinity Stress, Ascorbic Acid (AsA) and Gibberellic Acid (GA_3) on Seed Moisture Content (%) at 24 H after Germination of Wheat

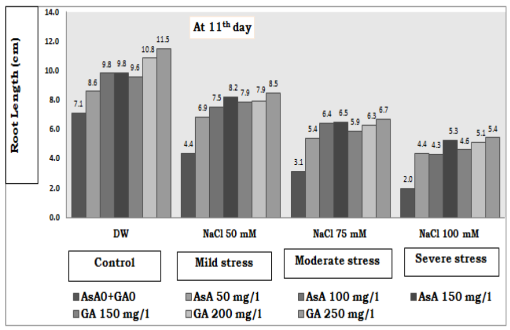

Figure 2: Effect of Nacl Induced Salinity Stress, Ascorbic Acid (AsA) and Gibberellic Acid (GA_3) on Root Length (Cm) at 11th Day after Germination of Wheat

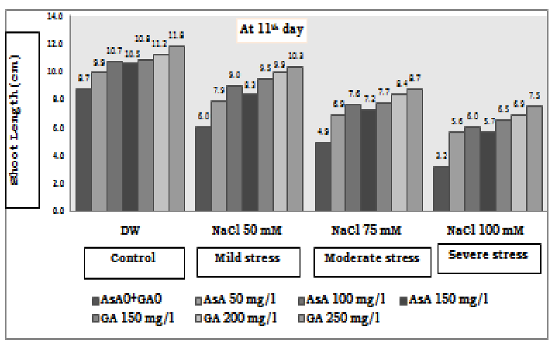

Figure 3: Effect of Nacl Induced Salinity Stress, Ascorbic Acid (AsA) and Gibberellic Acid (GA_3) on Shoot Length (Cm) at 11th Day after Germination of Wheat

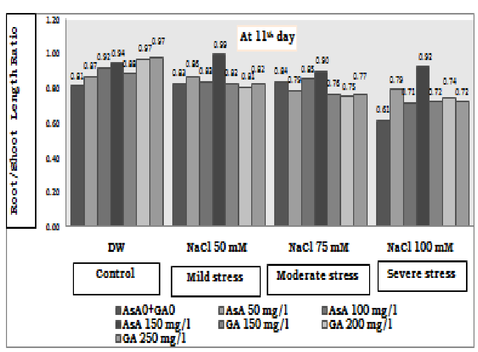

Figure 4: Effect of NaCl Induced Salinity Stress, Ascorbic Acid (AsA) and Gibberellic Acid (GA_3) on Root/Shoot Length Ratio at 11th Day after Germination of Wheat

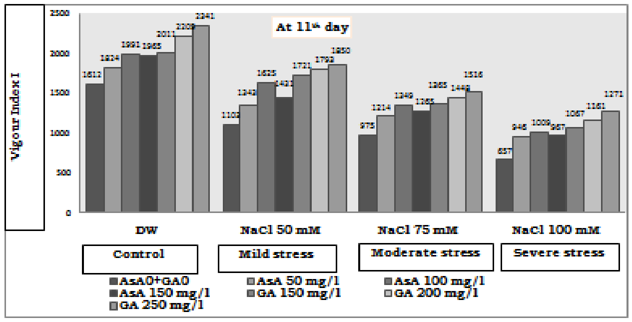

Figure 5: Effect of Nacl Induced Salinity Stress, Ascorbic Acid (Asa) and Gibberellic Acid (GA_3) on Vigour Index 1 at 11^{th} Day After Germination of Wheat

Vigour Index I

A progressive increase in salinity levels effectively reduced the vigour index I. NaCl, at the moderate and severe levels showed maximum reduction of (31%) and (34%) respectively as compared to mild stress (59%) levels. The result is supported by *Mosavian and Eshraghi (2013)* and *Elouaer et al (2012).* An increase in vigour index I was found after pretreatment of wheat seeds with ascorbic acid and gibberellic acid under saline condition. Application of AsA @ 100 mg L^{-1} was the best under all levels of salinity. The result is supported by *Ghoohestani et al (2012)* who reported that seeds priming of tomato with salicylic acid and ascorbic acid resulted in increased vigour index I under salt stress concentration. Ascorbic acid protects metabolic processes against H_2O_2 and other toxic derivatives of oxygen which affect many enzyme activities, minimizes the damage caused by oxidative processes through synergistic function with other antioxidants, and stabilizes membranes (*Agarwal and Pandey, 2004* and *Sairam et al, 2005*). All salinity levels under application of 250 mg L^{-1} GA_3 exhibited increased vigour index.

Proline Content

A sharp increase in proline content was found with increasing salinity concentrations. NaCl, at its moderate and severe stress level showed higher increase of 50% and 51% respectively in proline content as compared to and mild concentrations (41%). The result is in agreement with *Shamsi and Soheil (2013)* in wheat. Further increase in proline content was noticed after pretreatment of wheat seeds with ascorbic acid under saline condition. Application of highest concentration of AsA showed maximum increment. Similar result was proposed by *Azzedine et al (2011)* who reported that application of vitamin C was in durum wheat enhanced proline accumulation by triggering the biosynthesis of proline and other osmolytes. In reverse, gibberellic acid pretreatment showed reduction in the given parameter. The lowest concentration of GA_3 (150 mg L^{-1}) showed greater increment in proline content under all three stress levels. Similar result was reported by *Parvaneh and Hoseini (2015)* and *Anjali and Aruna (2013)* in wheat and spinach respectively. This may be due to increase in synthesis of protein at the expense of proline as a consequence of applied gibberellic acid.

Polyphenol Oxidase Activity

PPO activity increased considerably with increasing salinity levels. Slight induction (15%) in the given parameter was observed under mild salt stress level. However, at moderate and severe stress levels more than double increase of 36% and 38% were observed. Similar result was reported by *Sairam et al (2005)* in wheat. A noticeable increase in polyphenol oxidase activity was observed after pretreatment of wheat seeds with ascorbic acid and gibberellic acid under saline condition. Seeds pretreated with AsA @ 100 mg L^{-1} was numerically the best and treatment of AsA @ 150 mg L^{-1} was at

par with it under all three stress condition. The result is in agreement with *Elhamid et al (2014)* in wheat cultivar. AsA-induced enhanced salt tolerance in wheat plants was due to having a better antioxidant system as found in the present investigation for the effective removal of ROS plants, and maintenance of ion homeostasis. Application of the highest concentration GA_3 (250 mg L^{-1}) under all three stress conditions, showed maximum increase in PPO activity. Thus, it could be inferred that PPO plays a vital role in plant defense against oxidative stress by scavenging H_2O_2 in chloroplast, cytosol, mitochondria and peroxisome of plant cells. Among the two biostimulators, seeds treatment with AsA @ 100 mg L^{-1} was the best in ameliorating the adverse effect of salinity and increased polyphenol oxidase activity.

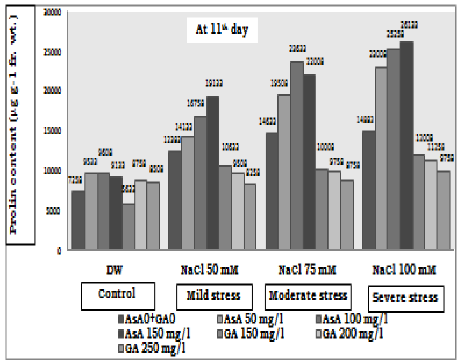

Figure 6: Effect of NaCl Induced Salinity Stress, Ascorbic Acid (AsA) and Gibberellic Acid (GA_3) on Proline Content at 11th Day after Germination of Wheat

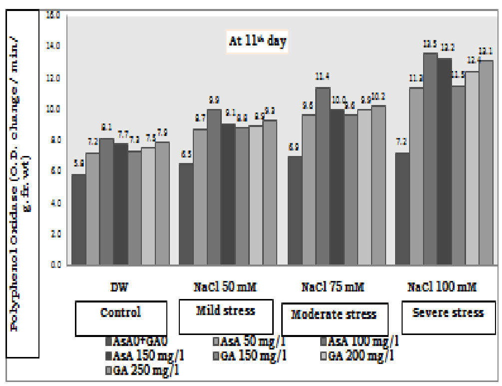

Figure 7: Effect of NaCl Induced Salinity Stress, Ascorbic Acid (AsA) and Gibberellic Acid (GA_3) on Polyphenol Oxidase at 11th Day after Germination of Wheat

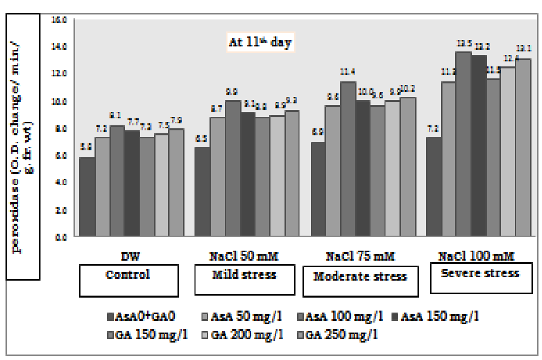

Figure 8: Effect of NaCl Induced Salinity Stress, Ascorbic Acid (AsA) and Gibberellic Acid (GA_3) on Peroxidase at 11th Day after Germination of Wheat

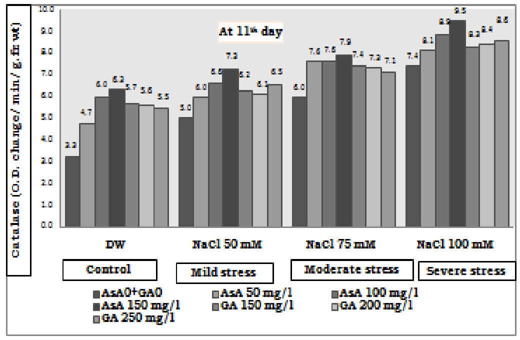

Figure 9: Effect of NaCl Induced Salinity Stress, Ascorbic Acid (AsA) and Gibberellic Acid (GA_3) on Catalase at 11th Day after Germination of Wheat

Peroxidase Activity

NaCl, at the severe stress level showed drastic increase of 19% as compared to its moderate and mild stress level with the induction of 15% and 9% respectively. This might be due to the toxic effects of the high turnover rate of H_2O_2 or its harmful ROS, which impair enzyme activities. Similarly, increased POD activity as an influence of salinity stress was reported by Dolatabadian *et al* (2009) in wheat. Pretreatment of wheat seeds with AsA and GA_3 caused a remarkable increase in POD activity under saline condition. Application AsA @ 100 mg L^{-1} was the best it under all three stress conditions. Similar result was reported by Elhamid *et al* (2014) in wheat and Hameda and Hanan (2015) in sweet peppers. However, under all three stress condition seeds treated with GA_3 @ 250 mg L^{-1} was the best in nullifying the adverse effect of salt stress. Younesi & Moradi (2014) in alfalfa and Mohammed (2007) in wheat found similar result. The probable region for such increase might be due to the role of GA_3 in triggering the synthesis of antioxidant enzyme under saline condition in response to oxidative damage through signal cascade. Considering the two, it was revealed that seeds treated with AsA @ 100 mg L^{-1} was the best in enhancing POD activity.

Catalase Activity

NaCl, at the severe stress level showed sharp increase of 56% in the given parameter as compared to its moderate and mild stress level with the increment to the tune of 45% and 35% respectively. Similar result of increased catalase activity when exposed to salt stress was reported in wheat by Esfandiari *et al* (2011), Marvi *et aI* (2011) and that Elhamid *et al* (2014). To be able to endure oxidative damage under salinity stress, plants triggered efficient antioxidant system and hence, increased the activity of CAT as an adaptive mechanism to reduce the H_2O_2 and offer protection against oxidative damage Agarwal and Pandey (2004). Pretreatment of wheat seeds with AsA and GA_3 caused a magnificent increase in CAT activity under saline condition. Among AsA treated seeds application of its highest concentration (150 mg L^{-1}) was numerically the best under all mild, moderate and severe stress conditions. Elhamid *et al* (2014), Farouk (2011) and Khan and Ashraf (2008) also reported similar result in wheat. The maximum catalase activity was exhibited by the application of the highest concentration of GA_3 (250 mg L^{-1}) under mild and severe stress conditions. However, under moderate stress condition treatment with GA_3 @ 150 mg L^{-1} caused maximum increase in catalase activity. Similar result was reported in wheat by Shaddad *et al* (2013), Tabatabaei (2013) and Parvaneh and Hoseini (2015) that catalase activity significantly increased in seeds primed with gibberellins as compared to the unprimed seeds under salt stress. Comparing the two, it was revealed that seeds treatment with AsA @ 150 mg L^{-1} was found most effective in enhancing catalase activity.

CONCLUSIONS

Thus, distinct favorable effect of ascorbic acid and gibberellic acid in alleviation of salinity stress could be discerned as evidenced by activated metabolism in terms of increased water and nutrient use efficiency, synthesis of osmolytes and antioxidants of germinating seedlings of wheat after pretreatment under saline condition. Hence, this study proves the role of these bio-stimulators in ameliorating the deleterious effect of salinity stress.

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